

Octyl and nonylphenol ethoxylates and carboxylates in wastewater and sediments by liquid chromatography/tandem mass spectrometry

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Abstract

This work presents an LC–MS–MS-based method for the quantitation of nonylphenol ethoxylates (NPEOs) and octylphenol ethoxylates (OPEOs) in water, sediment, and suspended particulate matter, and three of their carboxylated derivatives in water. The alkylphenol ethoxylates (APEOs) were analyzed using isotope dilution mass spectrometry with [¹³C₆]-labeled analogues, whereas the carboxylated derivatives were determined by external standard quantitation followed by confirmation using standard additions. The method was used to study APEO's behavior in a wastewater treatment plant (WWTP), where total dissolved NP0-16EO concentration was reduced by approximately 99% from influent (390 µg l⁻¹) to final effluent (4 µg l⁻¹), and total OP0-5EO concentration decreased by 94% from 3.1 to 0.2 µg l⁻¹. In contrast, the carboxylated derivatives were formed during the process with NP0-1EC concentrations increasing from 1.4 to 24 µg l⁻¹. Short-chain APEOs were present in higher proportions in particulate matter, presumably due to greater affinity for solids compared to the long-chain homologues. NP (0.49 µg l⁻¹) and NP0-1EC (4.8 µg l⁻¹) were the only APEO-related compounds detected in a surface water sample from a WWTP-impacted estuary; implying that 90% of the mass was in the form of carboxylated derivatives. Sediment analysis showed nonylphenol to be the single most abundant compound in sediments from the Baltimore Harbor area, where differences in homologue distribution suggested the presence of treated effluent in some of the sites and non-treated sources in the rest.

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1. Introduction

Alkylphenol ethoxylates (APEOs) are nonionic surfactants widely used in diverse cleaning and industrial applications (Talmage, 1994). Nonylphenol ethoxylates (NPEOs) and octylphenol ethoxylates (OPEOs) account for approximately 80% and 20% of the total APEO production (APE Research Council, 1999). After use, the APEOs reach wastewater treatment plants (WWTP) or water bodies, where biotransformation products are formed; these include the mono and diethoxylates, and carboxylated

derivatives (APECs) in aerobic environments, and the parent alkylphenols (APs), nonylphenol (NP) or octylphenol (OP), in anaerobic conditions (Montgomery-Brown and Reinhard, 2003). These transformation products are widespread aquatic pollutants (Bennie, 1999; Kolpin et al., 2002), especially in waters impacted by WWTP effluents, whose toxic and estrogenic properties (Servos, 1999) have raised concerns about their environmental fate. Traditionally, APEOs and their transformation products were analyzed by GC–MS and HPLC with fluorescence detection (HPLC-F), e.g., Ahel et al. (1987); Datta et al. (2002). Recently, LC–MS based methods have been developed to overcome intrinsic limitations of the analytical instrumentation used originally: GC–MS inability to analyze long-chain APEOs and HPLC-F relatively low selectivity. According to Lopez de Alda et al. (2003), few of these

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LC methods use tandem MS (possibly due to lack of instrumentation) even though it provides better selectivity and lower detection limits than single MS (Petrovic and Barceló, 2001). Additionally, most of the single and tandem MS methods tend to target the APs, and the short-chain APEOs and APECs (e.g., Ferguson et al., 2000; Petrovic et al., 2003; Jahnke et al., 2004; Loos et al., 2007). Methods for the long-chain NPEOs either exclude some of the lower molecular weight compounds, e.g., NP (Houde et al., 2002); require different columns for different sets of compounds (Martínez et al., 2004); use single MS (Shang et al., 1999; Ferguson et al., 2001); or do not resolve the different NPEO homologues in a mixture (Petrovic and Barceló, 2000). Furthermore, quantitative LC–MS analysis can be limited by suppression or enhancement of the analyte signal by matrix components (Ferguson et al., 2000). This problem can be circumvented using isotope dilution mass spectrometry (IDMS), which also allows simultaneous correction of analyte losses during sample extraction (Prichard et al., 1996), although it has the inherent disadvantage of the high cost and low availability of appropriate isotopic analogues (Sargent et al., 2002).

Previously, our group developed a quantitative LC–MS–MS method to analyze NP, OP, and the short-chain APEOs (AP1-5EO) in water and sediment (Loyo-Rosales et al., 2003). For the present work, a modification of these methods was introduced to employ IDMS quantitation using [$^{13}\text{C}_6$]-labeled analogues and to include the analysis of the long-chain NPEOs (NP6-16EO) in water and sediment, and the OP0-5EOs and NP0-16EOs in suspended particulate matter. At the same time, this method addressed some of the issues noted before, such as the use of more than one chromatographic column and the exclusion of NP from the analysis. A separate method was developed for the analysis of nonylphenoxyacetic acid (NP0EC), nonylphenoxyethoxyacetic acid (NP1EC), and octylphenoxyacetic acid (OP0EC) in water using external standard and standard additions quantitation; IDMS was not used for the APECs due to the lack of isotope-labeled analogues. These methods were used for the analysis of wastewater effluents from a large WWTP in the Mid-Atlantic region of the US and in sediments from the Baltimore Harbor area.

2. Materials and methods

2.1. Standards and reagents

Individual compounds were used as standards for the short-chain APEOs and the carboxylates, and they were either acquired from commercial sources –NP (Schenectady International, Schenectady, NY, USA; purity $\geq 95\%$), OP (Aldrich, Milwaukee, WI, USA; 97%), NP2EO (R&D product from Aldrich), NP0EC (R&D product, Huntsman Chemicals, Austin, TX, USA), NP1EC (Aldrich; 90%), and OP0EC (R&D product, Huntsman) – or purified in the laboratory from commercially-available

mixtures by flash chromatography as described previously (Datta et al., 2002; Loyo-Rosales et al., 2003) – NP1EO from Surfonic N-10 (Huntsman); NP3-5EO from POE(4) nonylphenol (Chem Service, West Chester, PA, USA); OP1-5EO from POE(3) and POE(5) *tert*-octylphenol (Chem Service). Purity of the isolated compounds was $>99\%$ with the exception of OP1EO (94%). Due to the lack of pure standards for the individual NP6-16EOs when this work was performed, previously characterized mixtures were used. Originally, Marlophen 810 (Chemische Werke, Hüls, Germany) characterized by Ahel et al. (2000) was used, but the presence of unreported OPEOs in the mixture resulted in an overestimation of the results by a factor of 1.7–2.8. Therefore, Surfonic N-95 (Schenectady International; $>97\%$) characterized by Huntsman, was chosen. A mixture of [$^{13}\text{C}_6$]NP (Cambridge Isotope Laboratories, Andover, MA, USA), [$^{13}\text{C}_6$]NP(1.6)EO and [$^{13}\text{C}_6$]NP-(9.5)EO – both synthesized by Ferguson et al. (2001) – in methanol was used for isotope dilution quantitation. Organic-free, high purity methanol, acetone, and dichloromethane (DCM) were acquired from Burdick and Jackson (Honeywell International, Muskegon, MI, USA). Carbon-free deionized water (DI water) was obtained using a NANOpure water purification system (Barnstead International, Dubuque, IA, USA). Other reagents included hydrochloric acid (Fisher Chemicals, Fair Lawn, NJ, USA), anhydrous sodium sulfate (Mallinckrodt Baker, Paris, KY, USA) and 99.99+ % purity ammonium acetate (Aldrich). Glassware was baked at 400 °C for 4 h in an industrial oven (Grieve, Round Lake, IL, USA) to avoid contamination with the analytes of interest, especially NP.

2.2. Sample collection

2.2.1. Water

Grab samples (~ 4 l) from a WWTP in the Mid-Atlantic region of the United States, and surface water from the Back River, MD were collected in July 2004 in previously-baked 1-gal amber glass bottles. WWTP sampling sites represented the different treatment stages: raw wastewater, primary, secondary, tertiary and final effluents. A surface water sample from Back River was provided by the MD Department of Natural Resources from a site east of Stansbury Point (NAD83 coordinates: 39.2834°, –76.4497°) (Maryland Department of Natural Resources, 2004) and downstream from a WWTP effluent discharge (not the same plant as above). One to 4 l of sample were filtered using a glass vacuum filter holder (Millipore, Billerica, MA, USA) with two pre-weighed glass fiber filters in series: GF/A 1.6 μm , and GF/F 0.7 μm (Whatman, Clifton, NJ, USA). Both filters and the filtration unit were previously baked 4 h at 400 °C. If necessary, filtered samples were stored overnight at 4 °C, and extraction occurred less than 24 h after filtration. The filters were dried overnight in the extraction hood, stored under vacuum, and weighed to calculate the amount of particulate matter in the samples.

2.2.2. Sediment

Sediment samples were collected from seven sites in the Baltimore Harbor area in October 1999 (Fig. 1). Exact locations were: Back River 1 (BR1): 39.2730°, –76.4419°; Back River 2 (BR2): 39.2450°, –76.4311°; Bear Creek 1 (BC1): 39.2585°, –76.4777°; Bear Creek 2 (BC2): 39.2272°, –76.4999°; Inner Harbor (IH): 39.2783°, –76.5931°; Gwynn's Falls (GF): 39.2615°, –76.6219°; White Rock (WR): 39.1730°, –76.4863°. The procedure for sediment collection was reported previously (Loyo-Rosales et al., 2003). Briefly, a ponar dredge was used to collect four to six grabs of sediment per site. The top 2 cm of each grab were removed, homogenized in a stainless steel container, poured into 250-ml pre-baked glass jars, and stored at –20 °C until extraction.

2.3. Extraction

2.3.1. Water

Extraction of APs and short-chain APEOs from water was performed as reported earlier (Loyo-Rosales et al., 2003), except that the [$^{13}\text{C}_6$]-labeled compounds were added to the samples before extraction. Briefly, water samples were spiked with 20 μl of the [$^{13}\text{C}_6$]-standard mix and extracted with solid-phase extraction (SPE) cartridges with a hyper-cross-linked hydroxylated poly(styrene–divinylbenzene) copolymer (SDB, Isolute ENV+, 500 mg, 6 ml, International Sorbent Technology, Hengoed, U.K.), which were then dried and eluted with 10 ml DCM, 10 ml methanol, and 12 ml acetone. The empty sample bottles were rinsed with 10 ml methanol, which was pooled with the elutes and reduced to 0.5 ml of methanol in a nitrogen evaporator (Organomation Associates, South Berlin, MA, USA); then, 0.5 ml DI water was added and the mixture filtered through an Acrodisc LC 13-mm syringe filter with a 0.2- μm PVDF membrane (Pall Gelman Laboratory, Ann

Arbor, MI, USA) using a 1-ml glass syringe (Hamilton, Reno, NV, USA). Both syringe and filter were rinsed with 0.5 ml of a 1 + 1 methanol/water mixture that was added to the extract; final volume was adjusted to 1.5 ml with clean methanol/water mixture when necessary. APECs were extracted from as follows: 500 ml of filtered water (pH adjusted to 2 with HCl) were extracted three times with 50 ml DCM each. DCM was exchanged to methanol in a rotary evaporator and reduced to 0.5 ml in a nitrogen evaporator, after which 0.5 ml of DI water was added. The extract was then processed as above.

2.3.2. Particulate matter

APs and APEOs in the particulate matter retained by the filters were extracted as follows: the dry filters were spiked with 20 μl of the [$^{13}\text{C}_6$]-standard mix and then Soxhlet extracted with 150 ml methanol for 8 h. Extracts were reduced to 0.5 ml by rotary and nitrogen evaporation and then processed as described in Section 2.3.1.

2.3.3. Sediment

APs and APEOs were extracted from sediments as described in Loyo-Rosales et al. (2003); except that addition of the [$^{13}\text{C}_6$]-labeled compounds occurred prior to extraction. Briefly, sediments were thawed, homogenized and air-dried. Approximately 1 g of dry sediment was mixed with sodium sulfate, spiked with 20 μl of the [$^{13}\text{C}_6$]-standard mix, and extracted by accelerated solvent extraction in an ASE 200 (Dionex, Sunnyvale, CA, USA) using hexane and acetone. These extracts were exchanged to hexane and cleaned using an SPE procedure with amino-propyl cartridges. After clean-up, the extracts were exchanged to 0.5 ml methanol and then processed as in Section 2.3.1.

2.4. LC–MS–MS Analysis

2.4.1. Ethoxylates

The chromatographic and mass spectrometry conditions for AP and APEO analysis were published previously (Loyo-Rosales et al., 2003). Briefly, separation was performed at 60 °C in a Waters 2690 XE separations module (Waters, Milford, MA, USA) with a 150 \times 4.6 mm MSPak GF-310 4D column (Shodex, Shoko, Tokyo, Japan). Mobile phase was 10 mM ammonium acetate in 50:50 v/v methanol/water and was gradually changed to 100% methanol. Tandem mass spectrometry analysis was done in a Quattro LC triple quadrupole mass spectrometer (Micromass, Manchester, UK) with an electrospray ionization source. The different compounds were analyzed using multiple-reaction monitoring (MRM); APEOs in electrospray positive mode (ESI+) and NP and OP in electrospray negative (ESI–). MS conditions for NP6-16EO and [$^{13}\text{C}_6$]NP5-15EOs are presented in Table 1. Parameters for NP, OP, [$^{13}\text{C}_6$]NP, NP1-5EO, OP1-5EO, and [$^{13}\text{C}_6$]NP1-4EO are available in Loyo-Rosales et al. (2003). Concentrations were calculated using the respective

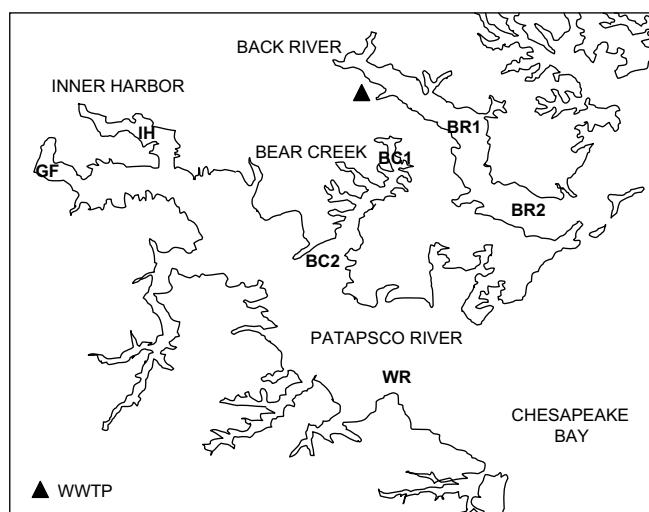


Fig. 1. Map of the Baltimore Harbor region showing approximate locations of the sediment sampling sites. BC: Bear Creek; BR: Back River; GF: Gwynn's Falls; IH: Inner Harbor; WR: White Rock.

Table 1
Ions and MS–MS parameters for NP6-16EO, NP0EC, NP1EC and OP0EC quantitation

Compound	Parent ion (<i>m/z</i>)	Fragment ion (<i>m/z</i>)	Retention time (min)	Cone (V)	Collision (eV)	Ion mode
NP6EO	502.3	359.1	18.2	20	18	ESI+
NP7EO	546.3	403.1	17.4	25	18	ESI+
NP8EO	590.3	573.5	16.7	30	18	ESI+
NP9EO	634.3	617.3	16.0	30	20	ESI+
NP10EO	678.3	661.3	15.3	35	20	ESI+
NP11EO	722.3	705.3	14.7	35	20	ESI+
NP12EO	766.3	749.5	14.1	35	22	ESI+
NP13EO	810.3	793.7	13.4	40	22	ESI+
NP14EO	854.3	837.6	12.8	40	24	ESI+
NP15EO	898.3	881.6	12.1	45	24	ESI+
NP16EO	942.6	925.8	11.4	45	26	ESI+
[¹³ C ₆]NP5EO	464.1	321.1	19.3	20	18	ESI+
[¹³ C ₆]NP6EO	508.3	365.1	18.2	20	18	ESI+
[¹³ C ₆]NP7EO	552.3	409.1	17.4	25	18	ESI+
[¹³ C ₆]NP8EO	596.3	579.5	16.7	30	18	ESI+
[¹³ C ₆]NP9EO	640.3	623.3	16.0	30	20	ESI+
[¹³ C ₆]NP10EO	684.3	667.3	15.3	35	20	ESI+
[¹³ C ₆]NP11EO	728.3	711.3	14.7	35	20	ESI+
[¹³ C ₆]NP12EO	772.3	755.5	14.1	35	22	ESI+
[¹³ C ₆]NP13EO	816.3	799.7	13.4	40	22	ESI+
[¹³ C ₆]NP14EO	860.3	843.6	12.8	40	24	ESI+
[¹³ C ₆]NP15EO	904.3	887.6	12.1	45	24	ESI+
NP0EC	277.5	219.0	10.2	30	20	ESI–
NP1EC	321.4	219.0	10.7	20	15	ESI–
OP0EC	263.3	205.0	9.2	30	20	ESI–

Parent ions correspond to $[M+NH_4]^+$ in ESI(+), and to $[M-H]^-$ in ESI(–). Fragment ions for NP6-7EO correspond to $[C_6H_5-(OCH_2CH_2)_x-OH + H]^+$, where $x = 6-7$; to $[M+H]^+$ for NP8-16EO, to $C_9H_{19}-C_6H_5-O^-$ for NP0-1EC and to $C_8H_{17}-C_6H_5-O^-$ for OP0EC.

[¹³C₆]-labeled compounds as internal standard. Eight calibration points were prepared in a 50:50 methanol/water mixture; concentrations ranged from 1–110 ng ml^{–1} for NP16EO to 12–1200 ng ml^{–1} for NP9EO. Peak integration and quantitation were performed automatically using MassLynx 4.0 (Micromass).

2.4.2. Carboxylates

APECs were analyzed with the same instruments and chromatographic column as above under similar conditions. The differences were that chromatographic separation was isocratic (20 min), and the entire run was done in ESI–. Specific parameters are presented in Table 1. Concentrations were initially calculated using an external standard calibration curve with eight points ranging from 6.6 to 660 ng ml^{–1}, and confirmed by standard additions adding successively a mixture of the compounds that doubled and tripled the concentrations calculated originally.

2.5. Quality assurance and quality control

Instrumental limits of detection (LODs) were calculated as the amount of the compound analyzed in the LC–MS–MS producing a signal-to-noise ratio of 3. Theoretical method detection limits (MDL-t) were calculated using LOD and normalizing for original sample amount and final volume of the extract. These values are compared to

method detection limits obtained from real samples (MDL-m) in Table 2. Limits of quantitation (LOQs) were set at five times the MDL-m value or at the lowest point of the calibration curve, whichever was higher. Every batch of up to 11 samples included at least one procedural blank. During the analysis by LC–MS–MS, the calibration curves were injected every 8–12 samples; r^2 values from linear regression analysis were expected to be at least 0.995 and the residual values for each calibration point below 10%.

3. Results and discussion

3.1. Analytical methods

3.1.1. Long-chain APEOs in water and sediment

These methods were originally developed for the short-chain APEOs, yielding good recoveries for these compounds (Loyo-Rosales et al., 2003). In contrast, the long-chain APEOs were not recovered as efficiently; the larger the number of EO units, the lower the recovery: 71, 66, 64, 59, 52, 44, 43, 36, 30, 26, and 21% for NP6EO to NP16EO in water and 88, 86, 83, 77, 67, 50, 33, 19, and 11% for NP6EO to NP14EO in sediment, respectively (NP15EO and NP16EO were not recovered from sediment in this case). The reasons behind these losses were not investigated thoroughly, but they are probably due to an increase of the compounds' affinity for materials such as

Table 2
Performance parameters for water and sediment methods

Compound	LOD (pg)	Water		Sediment	
		MDL-t (ng l ⁻¹)	MDL-m (ng l ⁻¹)	MDL-t (ng g ⁻¹)	MDL-m (ng g ⁻¹)
NP6EO	1.6	0.06	13	0.2	1.0
NP7EO	2.8	0.10	11	0.4	1.2
NP8EO	1.0	0.04	5	0.1	0.8
NP9EO	1.7	0.06	6	0.3	0.9
NP10EO	1.7	0.06	8	0.3	1.4
NP11EO	2.3	0.09	10	0.3	1.3
NP12EO	2.2	0.08	17	0.3	3.7
NP13EO	4.7	0.18	20	0.7	5.2
NP14EO	5.9	0.22	22	0.9	8.1
NP15EO	8.0	0.30	25	1.2	n.r.
NP16EO	8.2	0.31	29	1.2	n.r.
NP0EC	17	0.63	5	–	–
NP1EC	22	0.81	13	–	–
OP0EC	7.3	0.27	2	–	–

n.r.: not recovered.

LOD: instrumental limit of detection; MDL-t: theoretical method detection limit; MDL-m: method detection limit in matrix.

glass and the SPE cartridges or the sediment particles. Although good recoveries for the long-chain APEOs have been reported (Houde et al., 2002) using graphitized non-porous carbon (GCB); SDB was used in this case because it allowed a rapid extraction of up to 4 l of water – GCB cartridges tend to show high resistance to water flow (Rodríguez et al., 2000) – and provided reasonably cleaning of interfering compounds (SDB was originally selected to extract the short-chain APEOs partially because it was hypothesized that its structure would allow a more selective extraction of the APEOs than GCB, which is a more general sorbent). More importantly, NP was adequately recovered from SDB (Loyo-Rosales et al., 2003), but not from GCB (Houde et al., 2002); this constituted a serious disadvantage for the GCB method because of the high relevance of NP for the assessment of endocrine disruption potential. In order to offset the low recoveries of the long-chain APEOs from SDB cartridges, the IDMS approach was adopted.

As it was the case for the short-chain APEOs (Loyo-Rosales et al., 2003), the mixed-mode column allowed the chromatographic separation of the long-chain APEOs. Although peak resolution is not essential for the determination of different analytes when using tandem MS (the peaks can be resolved spectrometrically), chromatographic separation is advantageous because it decreases competition between coeluting compounds, which might result in ion suppression and lower sensitivity (Houde et al., 2002). Also similarly to the short-chain APEOs (Loyo-Rosales et al., 2003), LOD values for the long-chain APEOs were in the order of picograms (Table 2). LODs tended to increase with the number of EO units due to the increasing resistance of the compounds to fragment. APEOs were detected in this MS–MS method by forming $[M + NH_4]^+$ adducts and analyzing one of their fragments or the transition $[M + NH_4]^+ > [M + H]^+$, usually the most abundant in order to achieve maximum sensitivity. How-

ever, the stability of these adducts increases as the length of the ethoxylate chain increases, resulting in less fragmentation and lower sensitivity. In the case of the short-chain APEOs and NP6-7EO, fragments are produced with relative abundance and these were used for quantitation; for NP6-7EO, the fragments corresponded to the phenol-ethoxylate $[C_6H_5-(OCH_2CH_2)_x-OH + H]^+$, where $x = 6-7$ (Loyo-Rosales et al., 2003). Whereas for the NPnEOs with $n > 7$, fragments are not as abundant as their $[M + H]^+$ ions; therefore, the transition $[M + NH_4]^+ > [M + H]^+$ was used for quantitation in this case. This phenomenon also explains the low LOD values for NP9-12EO compared to the values for NP6-7EO.

The low LOD values observed would predict sub-part-per-trillion detection limits (MDL-t) for the water method, and sub-part-per-billion in sediment (Table 2). The presence of matrix compounds, however, resulted in detection limits (MDL-m) in the low part-per-trillion range in water and low part-per-billion in sediment. When compared to values in sediment, MDL-m in water increased much more with respect to MDL-t due to the much higher concentration factor involved, which resulted in higher amounts of matrix compounds and lower signal-to-noise ratios. The MDL-t values for the long-chain APEOs in water reported here are 1–2 orders of magnitude lower than those reported by Houde et al. (2002), mainly because in our approach a larger amount of sample was extracted – up to 4 l versus 100 ml. MDL-m values for the same compounds in sediment are comparable to those reported by Ferguson et al. (2001), but our approach offers the added selectivity of MS–MS.

3.1.2. Ethoxylates in particulate

For APEO extraction from the filters, three different solvents were investigated: acetonitrile, hexane:acetone 50:50, and methanol. Although the APEOs were better extracted with the hexane:acetone mixture, methanol was chosen

because it improved NP and OP recovery, which was very poor with the mixture. In contrast to the sediments, where recoveries for the long-chain APEOs were low, recovery of NP0-16EO from spiked filters (no matrix present) ranged from 73% to 100% (RSD = 9–19%, $n = 4$), and it improved with increasing number of EO units. However, when particulate was present in the filters, recovery values increased by a factor of ~ 2 due to matrix-induced ionization enhancement; an effect that was previously documented by Ferguson et al. (2001) in sediment extracts. IDMS was used to correct these effects.

3.1.3. Carboxylates in water

Although it would be ideal to obtain all the compounds of interest in a single extraction, the SPE method used for the ethoxylates did not perform well with the carboxylates, presumably because acidification of the water prevents the ENV+ solid-phase from retaining the analytes, as discussed previously (Loyo-Rosales et al., 2003). Therefore, liquid–liquid extraction with DCM was used. Recoveries from spiked DI water were 93, 93 and 94% for NP0EC, NP1EC and OP0EC, respectively (RSD = 16, 19, 15%; $n = 4$). LODs and MDLs for these compounds are presented in Table 2. These compounds tend to have higher LODs than the ethoxylates, suggesting that the LC–MS–MS is less sensitive to them. MDL-t for NP0EC and NP1EC are approximately one order of magnitude lower than those reported by Houde et al. (2002), and 2 (for NP0EC) to 6 (for NP1EC) times higher than Petrovic et al. (2003); the reason for these differences was most probably the magnitude of the concentration factors, which were higher in Petrovic et al. (2003) and lower in Houde et al. (2002). Ions, fragments, and MS conditions used for the carboxylates identification are listed in Table 1. In this case, the parent ions correspond to the $[M-H]^-$ quasi-molecular ions, and the fragments used for MS–MS detection correspond to the octyl- or nonyl-phenolate, which were abundantly produced. Houde et al. (2002) reported the same fragmentation pattern for NP0EC and NP1EC. Comparison of the concentrations obtained using external standard quantitation to those using standard additions indicated little or no matrix effects on APEC quantitation in any of the samples, either raw wastewater, the different treatment effluents, or in the Back River sample. For NP0EC and NP1EC concentrations obtained by both methods were virtually identical, whereas for OP0EC, concentrations calculated with external standard quantitation were consistently around 20% higher than the concentrations obtained by standard additions, suggesting a slight matrix enhancement effect for OP0EC.

3.2. Method application

3.2.1. Water

The methods described above were used to analyze wastewater and surface water from a WWTP and the Back River, MD, respectively. Results for the plant are presented

in Figs. 2–4. Fig. 2a shows the concentrations of dissolved NP0-16EO along the different treatment stages. Total dissolved NP0-16EO concentration was reduced by approximately 99%, from $390 \mu\text{g l}^{-1}$ in the raw wastewater to $4 \mu\text{g l}^{-1}$ in the final effluent. Moreover, the relative composition of the homologue mixture was enriched in the short-chain APEOs as the treatment progressed. Such phenomena are in agreement with previous observations that APEO degradation proceeds by a shortening of the ethoxylate chain (Ahel et al., 1994), which results in the formation of the short-chain APEOs. As a consequence, removal of NP, NP1EO and NP2EO at 85% was lower than total NPEO removal.

Due to their affinity for organic matter, the APEOs accumulate in suspended solids. The shorter the ethoxylate chain, the more hydrophobic the compound (Ahel and Giger, 1993). Therefore, the short-chain APEOs have a greater affinity for particulate matter, as Fig. 2b exemplifies. The concentration profile of the homologues in the solid phase was similar to the profile of the dissolved compounds, except that the short-chain APEOs were present in higher proportions. In fact, in raw wastewater and the primary effluent, more than 60% of the NP, NP1EO and NP2EO occurred in the particulate phase. This situation, combined to a solids removal during the process of more than 99%, was reflected in a 93% removal of the three compounds when considering both the dissolved and solid phases, in contrast with 85% when considering only the dissolved phase.

The OPEOs behaved similarly to the NPEOs. Concentrations of the dissolved OP0-5EOs are presented in Fig. 3. The individual OP homologues were present in the raw wastewater in concentrations that were 10–30 times lower than the respective NPEOs, reflecting volume differences in the use of these surfactants, which is dominated by the NPEOs (Ferguson et al., 2000). The total concentration of OPEOs almost doubled in the secondary effluent with respect to the raw wastewater, presumably as a product of higher ethoxylate degradation. The OPEOs where $n > 5$ were not quantified due to the lack of appropriate standards, but they were monitored qualitatively, and they disappeared gradually as the wastewater treatment progressed much like the NPEOs (data not shown).

In contrast to the APEOs, the concentration of the APECs increased along the treatment as can be seen in Fig. 4. In the final effluent, the amount of APECs present was approximately six times higher than the amount of the total ethoxylates, representing 85% of the compounds measured. Additionally, other carboxylated derivatives might be present that may increase the fraction of metabolites in the final effluent. Examples of these derivatives are long-chain APECs, and APEOs and APECs where the alkyl chain is also carboxylated, all of which have been previously reported in WWTP effluent and river water (Ding and Tzing, 1998; Di Corcia et al., 1998).

NP was found in the sample from Back River in a concentration of $0.49 \mu\text{g l}^{-1}$, whereas OP and the APEOs were

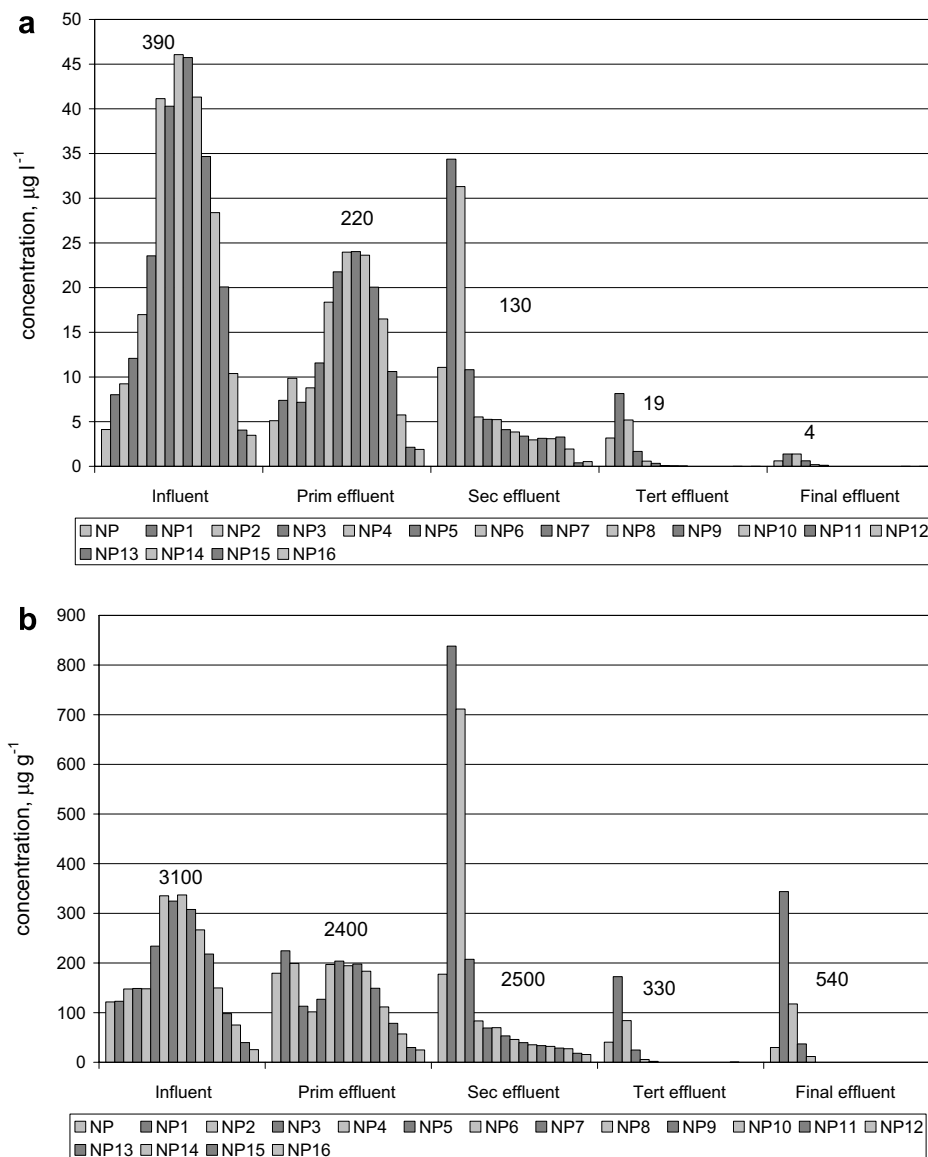


Fig. 2. Nonylphenol ethoxylate concentrations in a Mid-Atlantic region WWTP in (a) the aqueous phase, and (b) particulate matter. Numbers above the bars indicate total NP0-16EO concentrations ($\mu\text{g l}^{-1}$ in the aqueous phase and $\mu\text{g g}^{-1}$ in the particulate) in every treatment stage.

not detected. In contrast, APEC concentrations were 1.2, 3.6, and $0.056 \mu\text{g l}^{-1}$ for NP0EC, NP1EC, and OP0EC, respectively. Therefore, 90% of the mass of APEO-related compounds detected was in the form of transformation products, illustrating the importance of this type of compounds in the study of the environmental fate of organic chemicals. It also suggests that APECs might be better indicators for the presence of WWTP effluent in water than the APEOs.

3.2.2. Sediment

Results for NPEOs in sediments from the Baltimore Harbor area are shown in Fig. 5. As observed previously (Loyo-Rosales et al., 2003), NP tends to be the most abundant compound due at least in part to its higher affinity to solids. The sites with the highest concentrations of NPEOs were in Bear Creek and Back River, presumably

because of the presence of WWTP effluent in both bodies of water. These were followed by the sites in the Inner Harbor and Gwynn's Falls; whereas White Rock showed the lowest amounts of NPEOs, as expected from its location further away from wastewater effluent or other sources for these compounds. It is worthwhile noticing that the samples from Bear Creek and Back River contained a higher proportion of the short-chain APEOs than the rest of the samples; and that the concentrations of the long-chain APEOs in samples from the Inner Harbor and Gwynn's Falls seemed to increase with EO number, instead of leveling off and decrease, resembling the concentration profile of the most commonly used APEO mixtures. These observations are consistent with the presence of a treated effluent in Bear Creek and Back River, and suggest a non-treated source of NPEOs in the rest of the sites.

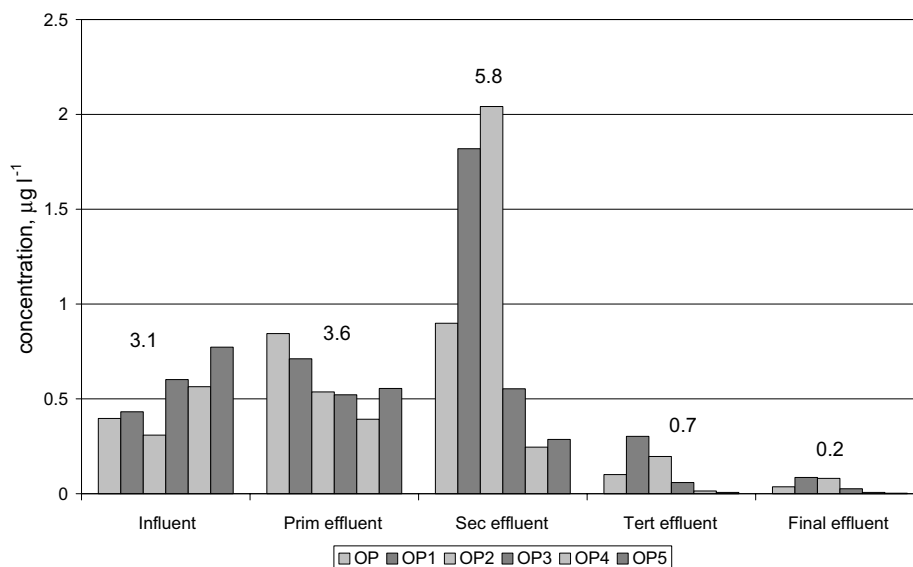


Fig. 3. Octylphenol ethoxylate concentrations in a Mid-Atlantic region WWTP. Numbers above the bars indicate total OP0-5EO concentrations ($\mu\text{g l}^{-1}$) in every treatment stage.

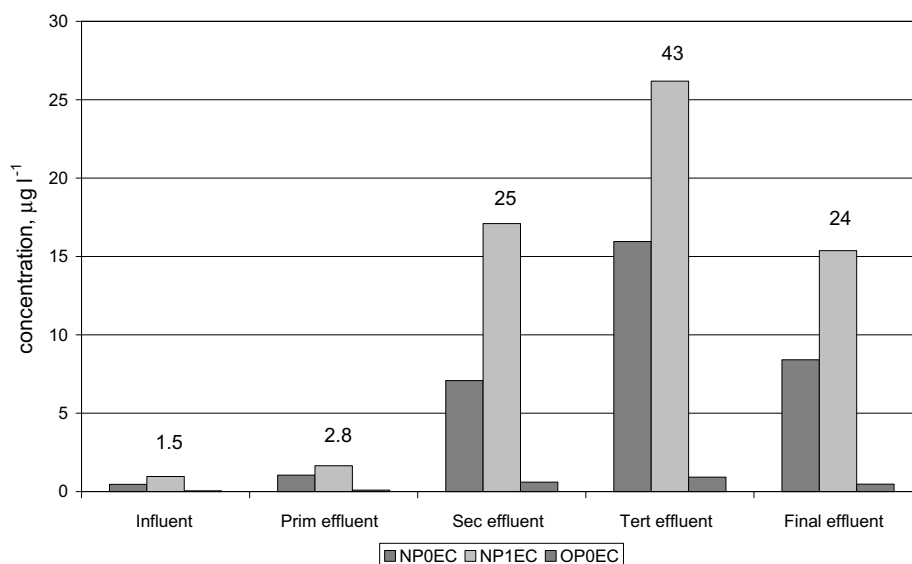


Fig. 4. Alkylphenol carboxylate concentrations in a Mid-Atlantic region WWTP (values obtained using external standard quantitation). The numbers above the bars indicate the total concentration ($\mu\text{g l}^{-1}$) of NP0EC, NP1EC, and OP0EC in every treatment stage.

OP and the OPEOs were also detected in these sediment samples, albeit in lower concentrations – 6–17 times less concentrated than their respective NPEOs. Their relative abundance paralleled that of the NPEOs, with OP being the most abundant compound, and Bear Creek and Back River the sites with the highest concentrations.

Short-chain APEO concentrations found in the Baltimore Harbor area sediments are within the ranges observed by Ferguson et al. (2001b) in Jamaica Bay, which is also a heavily urbanized estuary impacted by wastewater treatment plant effluents. There are few reports of long-chain APEOs in North American sediments; the concentrations presented here are in the same order of magnitude as those reported by Ferguson et al. (2001) in Jamaica Bay,

and by Shang et al. (1999) in the Strait of Georgia, British Columbia.

4. Conclusions

The methods presented in this article allow for the simultaneous extraction and quantitation of NP0-16EO and OP0-5EO present at environmentally-relevant concentrations in the dissolved and particulate fractions of WWTP effluents and surface water, as well as in sediments. The use of IDMS for quantitation of these compounds compensates for possible matrix effects and for analyte losses during sample extraction and processing. Unfortunately, isotopic-labeled analogues for the APECs are not

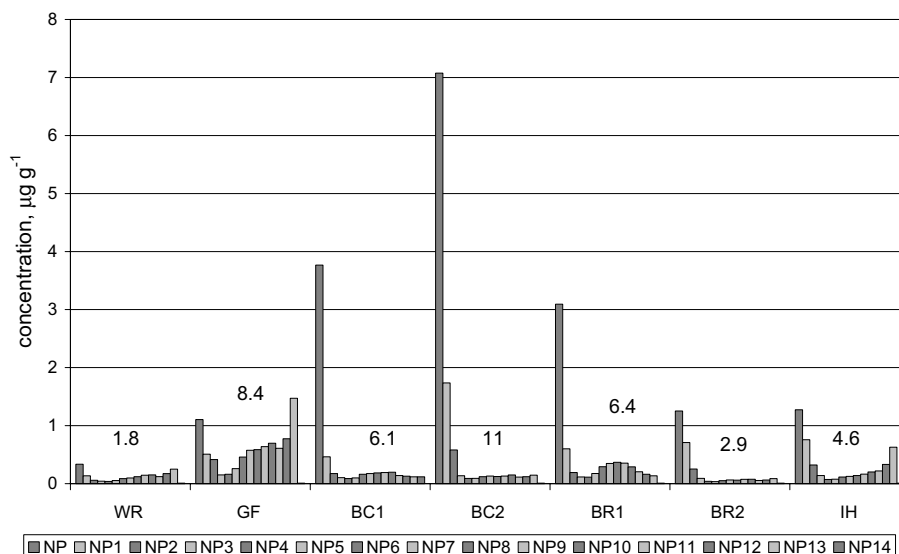


Fig. 5. Nonylphenol ethoxylate concentrations in sediments from the Baltimore Harbor area. The different sites are identified in Fig. 1. The numbers above the bars indicate total NP0-14EO concentrations ($\mu\text{g g}^{-1}$) in each site.

available, precluding the use of IDMS for the quantitation of these compounds. Together with the lack of individual long-chain APEOs, this lack of appropriate standards constitutes one of the main difficulties in conducting studies on the environmental fate of compounds that are subject to biotransformation. This work also illustrates the importance of measuring transformation products as well as the parent compounds in this kind of studies, not only because the former may be present in larger amounts, but also because their relative concentrations might be used as a tool for effluent source identification. A more detailed discussion on the fate of the APEOs within WWTPs and receiving waters will be presented in a future report.

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References

- Ahel, M., Giger, W., 1993. Partitioning of alkylphenols and alkylphenol polyethoxylates between water and organic solvents. *Chemosphere* 26, 1471–1478.
- Ahel, M., Conrad, T., Giger, W., 1987. Persistent organic chemicals in sewage effluents. 3. Determinations of nonylphenoxy carboxylic acids

- by high-resolution gas chromatography/mass spectrometry and high-performance liquid chromatography. *Environ. Sci. Technol.* 21, 697–703.
- Ahel, M., Giger, W., Koch, M., 1994. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-I. Occurrence and transformation in sewage treatment. *Water Res.* 28, 1131–1142.
- Ahel, M., Giger, W., Molnar, E., Ibric, S., 2000. Determination of nonylphenol polyethoxylates and their lipophilic metabolites in sewage effluents by normal-phase high-performance liquid chromatography and fluorescence detection. *Croat. Chem. Acta* 73, 209–227.
- APE Research Council, 1999. White Paper. <<http://www.aperc.org/docs/whitepaper-overview.html>> (accessed 08.05.06).
- Bennie, D.T., 1999. Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates. *Water Qual. Res. J. Canada* 34, 79–122.
- Datta, S., Loyo-Rosales, J.E., Rice, C.P., 2002. A simple method for the determination of trace levels of alkylphenolic compounds in fish tissue using pressurized fluid extraction, solid phase cleanup, and high-performance liquid chromatography fluorescence detection. *J. Agric. Food Chem.* 50, 1350–1354.
- Di Corcia, A., Costantino, A., Crescenzi, C., Marinoni, E., Samperi, R., 1998. Characterization of recalcitrant intermediates from biotransformation of the branched alkyl side chain of nonylphenol ethoxylate surfactants. *Environ. Sci. Technol.* 32, 2401–2409.
- Ding, W.-H., Tzing, S.-H., 1998. Analysis of nonylphenol polyethoxylates and their degradation products in river water and sewage effluent by gas chromatography-ion trap (tandem) mass spectrometry with electron impact and chemical ionization. *J. Chromatogr. A* 824, 79–90.
- Ferguson, P.L., Iden, C.R., Brownawell, B.J., 2000. Analysis of alkylphenol ethoxylate metabolites in the aquatic environment using liquid chromatography–electrospray mass spectrometry. *Anal. Chem.* 72, 4322–4330.
- Ferguson, P.L., Iden, C.R., Brownawell, B.J., 2001. Analysis of nonylphenol and nonylphenol ethoxylates in environmental samples by mixed-mode high-performance liquid chromatography–electrospray mass spectrometry. *J. Chromatogr. A* 938, 79–91.
- Ferguson, P.L., Iden, C.R., Brownawell, B.J., 2001b. Distribution and fate of neutral alkylphenol ethoxylate metabolites in a sewage-impacted urban estuary. *Environ. Sci. Technol.* 35, 2428–2435.
- Houde, F., DeBlois, C., Berryman, D., 2002. Liquid chromatographic-tandem mass spectrometric determination of nonylphenol polyethoxylates and nonylphenol carboxylic acids in surface water. *J. Chromatogr. A* 961, 245–256.

- Jahnke, A., Gandrass, J., Ruck, W., 2004. Simultaneous determination of alkylphenol ethoxylates and their biotransformation products by liquid chromatography/electrospray ionisation tandem mass spectrometry. *J. Chromatogr. A* 1035, 115–122.
- Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ. Sci. Technol.* 36, 1202–1211.
- Loos, R., Hanke, G., Umlauf, G., Eisenreich, S.J., 2007. LC–MS–MS analysis and occurrence of octyl- and nonylphenol, their ethoxylates and their carboxylates in Belgian and Italian textile industry, waste water treatment plant effluents and surface waters. *Chemosphere* 66, 690–699.
- Lopez de Alda, M.J., Díaz-Cruz, S., Petrovic, M., Barceló, D., 2003. Liquid chromatography–(tandem) mass spectrometry of selected emerging pollutants (steroid hormones, drugs and alkylphenolic surfactants) in the aquatic environment. *J. Chromatogr. A* 1000, 503–526.
- Loyo-Rosales, J.E., Schmitz-Afonso, I., Rice, C.P., Torrents, A., 2003. Analysis of octyl- and nonylphenol and their ethoxylates in water and sediments by liquid chromatography/tandem mass spectrometry. *Anal. Chem.* 75, 4811–4817.
- Martínez, E., Gans, O., Weber, H., Scharf, S., 2004. Analysis of nonylphenol polyethoxylates and their metabolites in water samples by high-performance liquid chromatography with electrospray mass spectrometry detection. *Water Sci. Technol.* 50, 157–163.
- Maryland Department of Natural Resources, 2004. Water Quality Monitoring Data. <http://mddnr.chesapeakebay.net/bay_cond/bay_cond.cfm?param=bdo&station=WT41> (accessed 25.11.04).
- Montgomery-Brown, J., Reinhard, M., 2003. Occurrence and behavior of alkylphenol polyethoxylates in the environment. *Environ. Eng. Sci.* 20, 471–486.
- Petrovic, M., Barceló, D., 2000. Determination of anionic and nonionic surfactants, their degradation products, and endocrine-disrupting compounds in sewage sludge by liquid chromatography/mass spectrometry. *Anal. Chem.* 72, 4560–4567.
- Petrovic, M., Barceló, D., 2001. Analysis of ethoxylated nonionic surfactants and their metabolites by liquid chromatography/atmospheric pressure ionization mass spectrometry. *J. Mass Spectrom.* 36, 1173–1185.
- Petrovic, M., Barceló, D., Diaz, A., Ventura, F., 2003. Low nanogram per liter determination of halogenated nonylphenols, nonylphenol carboxylates, and their non-halogenated precursors in water and sludge by liquid chromatography electrospray tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* 14, 516–527.
- Prichard, E., MacKay, G.M., Points, J. (Eds.), 1996. *Trace Analysis: A Structured Approach to Obtaining Reliable Results*. Royal Society of Chemistry, Cambridge, p. 280.
- Rodríguez, I., Llompart, M.P., Cela, R., 2000. Solid-phase extraction of phenols. *J. Chromatogr. A* 885, 291–304.
- Sargent, M., Harte, R., Harrington, C. (Eds.), 2002. *Guidelines For Achieving High Accuracy In Isotope Dilution Mass Spectrometry (IDMS)*. Royal Society of Chemistry, Cambridge, p. 3.
- Servos, M.R., 1999. Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. *Water Qual. Res. J. Canada* 34, 123–177.
- Shang, D.Y., Ikonou, M.G., Macdonald, R.W., 1999. Quantitative determination of nonylphenol polyethoxylate surfactants in marine sediment using normal-phase liquid chromatography–electrospray mass spectrometry. *J. Chromatogr. A* 849, 467–482.
- Talmage, S.S., 1994. *Environmental and Human Safety of Major Surfactants. Alcohol Ethoxylates and Alkylphenol Ethoxylates*. The Soap and Detergent Association and Lewis Publishers, Boca Raton, FL.